Amendments to the Specification:

Please cancel the paragraph at page 40, lines 14-26, and, in its place, add the following new paragraph:

A further aspect of the invention provides a peptide substrate for LKB1 comprising the amino acid sequence LSNLYHQGKFLQTFCGSPLY (SEQ ID NO:16) or FGNFYKSGEPLSTWCGSPPY (SEQ ID NO:17) or LSNMMSDGEFLRTSCGSPNY (SEQ ID NO:18) or MASLQVGDSLLETSCGSPHY (SEQ ID NO:19) or FSNEFTVGGKLDTFCGSPPY (SEQ ID NO:20) or AKPKGNKDYHLQTCCGSLAY (SEQ ID NO:21); or a said sequence with from one to four substitutions therein at any position other than the underlined residue and/or a conservative substitution at the underlined residue; or at least ten contiguous residues of a said sequence encompassing the underlined residue. The peptide substrate typically has less than about 30 or 25 residues, for example, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 residues. Within this range, longer peptides may have better affinity for the LKB1 complex, whilst shorter peptides may have better solubility in aqueous liquids.

Please cancel the paragraph bridging pages 40 and 41, and, in its place, add the following new paragraph:

In particular, the peptide substrate for LKB1 may consist of the amino acid sequence LSNLYHQGKFLQTFCGSPLY (SEQ ID NO:16) or LSNLYHQGKFLQTFCGSPLYRRR (SEQ ID NO:23) or SNLYHQGKFLQTFCGSPLY (SEQ ID NO:24) or SNLYHQGKFLQTFCGSPLYRRR (SEQ ID NO:25) or LSNLYHQGKFLQTFCGSPLY (SEQ ID NO:16) or LSNLYHQGKFLQTFCGSPLYRRR (SEQ ID NO:23) or FGNFYKSGEPLSTWCGSPPY (SEQ ID NO:17) or FGNFYKSGEPLSTWCGSPPYRRR (SEQ ID NO:29) or LSNMMSDGEFLRTSCGSPNY (SEQ ID NO:18) or LSNMMSDGEFLRTSCGSPNYRRR (SEQ ID NO:31) or MASLQVGDSLLETSCGSPHY (SEQ ID NO:19) or MASLQVGDSLLETSCGSPHYRRR (SEQ ID NO:33) or FSNEFTVGGKLDTFCGSPPY (SEQ ID NO:20) or FSNEFTVGGKLDTFCGSPPYRRR (SEQ ID NO:35) or AKPKGNKDYHLQTCCGSLAY (SEQ ID NO:21) or AKPKGNKDYHLQTCCGSLAYRRR (SEQ ID NO:37). The tripeptide RRR may be useful in anchoring the peptide on p81 membrane and may be replaced by a different anchoring moiety, for example if a different support is used.

Please cancel the paragraph bridging pages 41 and 42 and, in its place, add the following new paragraph:

A further aspect of the invention provides an antibody reactive with a peptide antigen having the amino acid sequence MVAGLTLGKGPESPDGDVS (SEQ ID NO:38) (residues 1-20 of human BRSK1), LSWGAGLKGQKVATSYESSL (SEQ ID NO:39) (residues 655-674 of human BRSK2), MEGAAAPVAGDRPDLGLGAPG (SEQ ID NO:40) (residues 1-21 of human NUAK1), TDCQEVTATYRQALRVCSKLT (SEQ ID NO:41) (residues 653-673 of human NUAK2), MVMADGPRHLQRGPVRVGFYD (SEQ ID NO:42) (residues 1-21 of human QIK), MVIMSEFSADPAGQGQGQQK (SEQ ID NO:43) (residues 1-20 of human SIK), GDCEMEDLMPCSLGTFVLVQ (SEQ ID NO:44) (residues 765-784 of human SIK), TDILLSYKHPEVSFSMEQAGV (SEQ ID NO:45) (residues 1349-1369 of human QSK), SGTSIAFKNIASKIANELKL (SEQ ID NO:46) (residues 776-795 of human MARK1), MSSRTVLAPGNDRNSDTHGT (SEQ ID NO:47) (residues 1-20 of human MARK4), MKDYDELLKYYELHETIGTG (SEQ ID NO:48) (residues 1-20 of human MELK), CTSPPDSFLDDHHLTR (SEQ ID NO:49) (residues 344-358 of rat AMPKa1), or CDPMKRATIKDIRE (SEQ ID NO:50) (residues 252 to 264 of rat AMPKa1). Such an antibody may be raised using the given peptide as immunogen, as well known to those skilled in the art. Alternative methods of obtaining such an antibody will also be well known to those

skilled in the art, for example using phage display techniques.

Please cancel the paragraph bridging pages 44 and 45, and, in its place, add the following new paragraph:

Figure 2. Amino acid sequence and tissue distribution patterns of MO25α and MO25β isoforms. (A) Amino acid sequence alignment of the human MO25α (NCBI accession number NP_057373) and MO25β (NCBI accession number Q9H9S4) isoforms as well as *C. elegans* MO25α (NCBI accession number CAB16486) and MO25β (NCBI accession number NP_508691) and *Drosophila* MO25 (NCBI accession number P91891) putative homologues. Conserved residues are boxed in black, and homologous residues are shaded in grey. Sequence alignments were performed using the CLUSTALW and BOXSHADE programmes at www.ch.embnet.org using standard parameters. (B) A ^{32}P-labelled fragment of the MO25α cDNA was used to probe a Northern blot containing polyadenylated RNA isolated from the indicated human tissues. The membrane was autoradiographed, and the MO25α probe was observed to hybridise to a 4.2-kb message, identical to the size predicted for the MO25α message from database analysis. As a loading

control, the Northern Blot was hybridised with a β-actin probe. (C &D) The indicated mouse tissue (C) or cell (D) extracts containing 20 μg of total cell protein were immunoblotted with the anti-MO25α and anti-MO25β antibodies.

Please cancel the paragraph bridging pages 46, 47, and 48, and, in its place, add the following new paragraph:

Figure 6. MO25a recognises the C-terminal 3 residues of STRADα. (A) N-terminal GST tagged wild type STRADα or the indicated mutants of STRADα were expressed in 293 cells together with Myc-MO25α, and 36 h post transfection the STRADα proteins were affinity purified from the cell lysates using glutathione-Sepharose. Similar amounts of the purified proteins were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting with anti-Myc antibody to detect co-purified Myc-MO25α, or with anti-GST antibody to ensure that comparable amounts of the GST tagged proteins were present in each lane (upper and middle panels). 5 μg of the total cell lysates prior to affinity purification were also subjected to immunoblotting with anti-Myc antibody to ensure that Myc-MO25α was expressed at similar levels in each condition (lower panel). (B) 0.5 mg of the indicated cell lysates

was incubated with 5 μg of an N-terminal biotinylated peptide encompassing either the C-terminal 12 residues STRADα conjugated to streptavidin-Sepharose (NLEELEVDDWEF (SEQ ID NO: 51), termed STRADα-C12) or mutants of this peptide in which the indicated residues were individually mutated to Ala. Following isolation and washing of the beads, the samples were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted with an anti-MO25α antibody. (C) Binding of bacterially expressed MO25α to the indicated peptides was analysed by surface plasmon resonance BiaCore analysis as described in Materials and Methods. Binding was analysed over a range of MO25α concentrations (6.25-3200 nM) and the response level at the steady-state binding was plotted versus the log of the MO25α concentration. The estimated K_d for the STRADα-C12 peptide was obtained by fitting the data to the formula [m1 X m0/(m0 + m2)] using Kaleidagraph software and the K_d was calculated to be 850 nM. WEF-C12 corresponds to NLEELEVDDWEF (SEQ ID NO:51), WEA-C12 corresponds to NLEELEVDDWEA (SEQ ID NO:52), AEF-C12 corresponds to NLEELEVDDAEF (SEQ ID NO:53), WAF-C12 corresponds to NLEELEVDDWAF (SEQ ID NO:54), WEF-C6 corresponds to VDDWEF (SEQ ID NO:55).

Please cancel the paragraph bridging pages 49 and 50, and, in its place, add the following new paragraph:

Figure 10. Amino acid sequence alignment of STRAD isoforms with closest STE20-kinase relatives. (A) Amino acid sequence alignment of the human STRADα (NCBI accession number AAG48269) and STRADβ (NCBI accession number AAM19143) with human SPAK (NCBI accession number AAC72238) and human OSR1 (NCBI accession number NP_005100). Conserved residues are boxed in black, and homologous residues are shaded in grey. The catalytic Asp (subdomain VI) and Asp-Phe-Gly (subdomain VII) present in the active SPAK and OSR1 protein kinases, but lacking in the STRADα and STRADβ pseudokinases are boxed and marked with an asterisk. The C-terminal Trp-Glu-Phe motif present in STRADα and STRADβ corresponding to a Phe-Glu-Phe sequence in SPAK and OSR1 are boxed and marked with triangles and the sites of LKB1 phosphorylation in STRADα (Thr329 and Thr419) (Baas et al., 2003) are marked with arrows. Sequence alignments were performed using the CLUSTALW and BOXSHADE programmes at www.ch.embnet.org using standard parameters.

Please cancel the paragraph at page 80, lines 7-15, and, in its place, add the following new paragraph:

Peptides. The biotinylated peptides STRADα-C12 (Biotin-C6spacer-NLEELEVDDWEF (SEQ ID NO:51)), and STRADα-C6 (Biotin-C6spacer-VDDWEF (SEQ ID NO:55)) whose sequences encompass the last 12, and 6 residues of STRADα respectively, the Flag peptide (DYKDDDDK (SEQ ID NO:97)), and peptides used to raise antibodies were synthesised by Dr G. Bloomberg (University of Bristol, UK). The biotinylated peptides encompassing the last 12 residues of STRADα in which each residue was individually replaced by an Alanine were synthesized using previously described methodology (Lizcano et al., 2002).

Please cancel the paragraph bridging pages 79 and 80, and, in its place, add the following new paragraph:

Antibodies. The LKB1 antibody used for immunoblotting was raised against the mouse LKB1 protein as described previously (Sapkota et al., 2001). As this antibody does not immunoprecipitate efficiently the human LKB1 protein, we raised an antibody in sheep against the human LKB1 protein expressed as a GST-fusion protein in *E. coli*, that efficiently immunoprecipitated human and rat LKB1. This antibody was used for all the immunoprecipitating experiments in this study. The anti-MO25α and anti-MO25β antibodies were

raised in sheep against the MO25α and MO25b human proteins expressed in *E. coli* as described below. The LKB1 and MO25 antibodies were affinity-purified on CH-Sepharose covalently coupled to the protein antigen used to raise the antibody. The monoclonal antibody recognising the STRADα protein has been described previously (Baas et al., 2003). The phospho-specific antibody recognizing MBP phosphorylated at Thr65 (T65-P) was raised in sheep against peptide GHHAARTTHYGSLPQ (SEQ ID NO:98) corresponding to residues 58-72 of bovine MBP in which the underlined residue is phosphothreonine. The antibody was affinity-purified on CH-Sepharose covalently coupled to the phosphorylated peptide and then passed through a column of CH-Sepharose coupled to the non-phosphorylated peptide. Antibody that did not bind to the latter column was selected. Monoclonal antibodies recognizing the GST and Flag epitope tags were obtained from Sigma, the monoclonal antibody recognizing the Myc epitope tag was purchased from Roche, and secondary antibodies coupled to horseradish peroxidase used for immunoblotting were obtained from Pierce. Preimmune IgG used in control immunoprecipitation experiments was obtained from preimmune serum employing Protein G-Sepharose.

Please cancel the paragraph bridging pages 82 and 83, and, in its place, add the following new paragraph:

DNA constructs. Constructs expressing the full length wild type and kinase dead mouse LKB1 have been described previously (Sapkota et al., 2002a; Sapkota et al., 2002b; Sapkota et al., 2001). In order to generate the N-terminal Myc-tagged MO25a cDNA, a PCR was performed with primers: 5'-ggatccgccaccatggagcagaagctgatctctgaa gaggacttgccgttcccgtttgggaagtctcacaaat-3' (SEQ ID NO:99) and 5'-ggatccttaagcttcttgctgagctggtctcttc -3' (SEQ ID NO:100) using an IMAGE Consortium EST clone (IMAGE clone 4822388, NCBI Acc. BG719459) as a template. The resulting PCR product was ligated into pCR2.1-TOPO vector (Invitrogen), and subcloned as a *Bam*H1- *Bam*H1 fragment into the pEBG-2T (Sanchez et al., 1994), pCMV5 (Anderson et al., 1989) and pGEX-6T (Amersham) vectors. To generate the N-terminal Myc-tagged MO25β cDNA, a PCR was performed with primers 5'-caccggatccgccaccatggagcagaagctgatctct gaagaggacttgcctttgtttagtaaatcacacaaaaatcc-3' (SEQ ID NO:101) and 5'- ggatcctcaaggggccgttttctt caagtctcgg-3' (SEQ ID NO:102) using an IMAGE Consortium EST clone (IMAGE 1958217, NCBI Acc. AI252223) as a template. The resulting PCR product was ligated into pENTR/D-TOPO Gateway intermediate vector (Invitrogen), and converted

into Gateway modified pEBG-2T, pCMV5 and pGEX-6T expression vectors. It should be noted that the human MO25β IMAGE clone we obtained encodes for an Ala instead of a Val at position 153, compared to the submitted sequence (NCBI accession number Q9H9S4). Sequence analysis of the nucleotide sequence of several independent MO25β sequences listed Table 1, indicated that all of these possess an Ala at position 153. Expression constructs for Flag-tagged STRADα were PCR amplified with primers 5'- ggatccgccacc atggactacaaggacgacgatgacaagtcatttcttgtaagtaaacca gagcgaatc-3' (SEQ ID NO:103) and 5'- ggatcctcaga actcccaatcgtccacctccagct-3 (SEQ ID NO:104) using a human STRADα cDNA as a template (Baas et al., 2003). The PCR product was ligated into pCR2.1-TOPO vector and subcloned as a *Bam*H1-*Not*1 fragment into pEBG-2T or as a *Bam*H1-*Xba*1 insert into pCMV5.

Please cancel the paragraph bridging pages 83 and 84, and, in its place, add the following new paragraph:

In order to clone STRADβ, two IMAGE EST clones (5301936 and 5501540), which together encompass the entire coding sequence were used as templates to generate full length flag-tagged STRADβ cDNA by PCR using primers 5'-ggatccgccacc atggactacaaggacgacgatgacaagtctcttttggattgcttctgc

acttcaag-3' (SEQ ID NO: 105) and 5'-ggatccctagaa ttcccagtatgagtctttttcatc-3' (SEQ ID NO: 106). The PCR product was ligated into pCR2.1-TOPO vector and subcloned as a *Bam*H1-*Bam*H1 fragment into the pEBG-2T and pCMV5 vectors. The catalytic fragment of mouse LKB1 (residues 44-343) possessing an N-terminal Flag epitope tag was PCR amplified from a mouse LKB1 cDNA (Sapkota et al., 2001) using the primers: 5'-actagtgccaccatggactacaaggacgacgatgacaagaagctcatc ggcaagtacctgatgggg-3' (SEQ ID NO: 107) and 5'-actagttcagtcctccaggtagggcactacagtcat-3' (SEQ ID NO: 108) and subcloned into pCMV5 vector as an *Spe*I-*Spe*I fragment. The construct encoding for the expression of GFP tagged human LKB1 with no other epitope tag was described previously (Boudeau et al., 2003b). Site directed mutagenesis was performed employing standard procedures using the Quickchange mutagenesis kit (Stratagene). Deletion mutations were generated by introducing STOP codons at the indicated positions.

Please cancel the paragraph at page 111, lines 15-29, and, in its place, add the following new paragraph:

Materials, proteins and antibodies

Protein G-Sepharose and prepacked Q-Sepharose columns were from Amersham Pharmacia Biotech,

UK. GST-AMPKα1-catalytic domain was expressed in *E. coli* and purified as described previously [44]. Sheep antibodies against the α1 and α2 subunits of AMPK [45], human LKB1, MO25α and MO25β (Example 1), and phosphospecific antibody against the Thr-172 site on the AMPK α subunit (anti-pT172) [46] were described previously. Sheep antibody against AMPKα1-catalytic domain was raised against the peptide CDPMKRATpIKDIRE (SEQ ID NO:109) (cysteine + residues 252-264 of rat α1, Tp = phosphothreonine) using methods described for anti-pT172 [46]. Although designed as a phosphospecific antibody, it recognizes GST-AMPKα1-catalytic domain expressed in bacteria and recognition is not affected by protein phosphatase treatment. The monoclonal antibody against STRADα was described previously [30]. Sources of other materials and proteins were described previously [14].

Please cancel the paragraph at page 120, line 7, and, in its place, add the following new paragraph:

41. **Kinase.com** [www.kinase.com]

Please cancel the paragraph at page 121, lines 10-11, and, in its place, add the following new paragraph:

50. BOXSHADE v3.21
[www.ch.embnet.org/software/BOX_form.html]

Please cancel the paragraph at page 121, lines 19-21, and, in its place, add the following new paragraph:

A kinase assay for LKB1 can be performed using a peptide substrate, for example using the T-loop peptide of AMPK whose sequence is LSNMMSDGEFLRTSCGSPNRRR (SEQ ID NO: 110) (HUMAN AMPKalpha-1 Residues 163-181 with 3 additional Arg residues added to the C-terminal to enable binding to P81 paper).

Please cancel the paragraph bridging pages 121 and 122, and, in its place, add the following new paragraph:

An assay may be performed as follows. Other assays using such a substrate peptide may be used, for example in a high throughput format. The standard assay (50 μl) contained: 0.3 μg of purified GST-LKB1-STRADa-MO25α complex, 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 0.1% (by vol) 2-mercaptoethanol, 10mM magnesium acetate, 0.1 mM [γ^{32}P]ATP (~200 cpm/pmol) and T-loop peptide

(LSNMMSDGEFLRTSCGSPRRR (SEQ ID NO: 110), 10-2000 μM). The assays were carried out for 30 min at 30°C, then terminated by pipetting the reaction mixture onto a 1 cm square of p81 paper, which was washed with 6 changes of 250 ml of 50 mM phosphoric acid (2 min each wash), the paper washed once in 200 ml of acetone and dried. Radioactivity bound to the p81 paper was quantitated by Cherenkov counting. 1 unit of activity is the amount of enzyme that catalyses the phosphorylation of 1 nmol of peptide per minute.

Please cancel the paragraph bridging pages 138 and 139, and, in its place, add the following new paragraph:

Antibodies.

The following antibodies were raised in sheep and affinity purified on the appropriate peptide antigen: BRSK1 (MVAGLTLGKGPESPDGDVS (SEQ ID NO:38), residues 1-20 of human BRSK1), BRSK2 (LSWGAGLKGQKVATSYESSL (SEQ ID NO:39), residues 655-674 of human BRSK2), NUAK1 (MEGAAAPVAGDRPDLGLGAPG (SEQ ID NO:40) residues 1-21 of human NUAK1), NUAK2 (TDCQEVTATYRQALRVCSKLT (SEQ ID NO:41), residues 653-673 of human NUAK2), QIK (MVMADGPRHLQRGPVRVGFYD (SEQ ID NO:42), residues 1-21 of human QIK), SIK (MVIMSEFSADPAGQGQGQQK

(SEQ ID NO:43), residues 1-20 of human SIK used for immunoprecipitation from human cells), SIK (GDCEMEDLMPCSLGTFVLVQ (SEQ ID NO:44), residues 765-784 of human SIK used for immunoprecipitation from mouse cells), QSK (TDILLSYKHPEVSFSMEQAGV (SEQ ID NO:45), residues 1349-1369 of human QSK), MARK1 (SGTSIAFKNIASKIANELKL (SEQ ID NO:46), residues 776-795 of human MARK1), MARK4 (MSSRTVLAPGNDRNSDTHGT (SEQ ID NO:47), residues 1-20 of human MARK4), MELK (MKDYDELLKYYELHETIGTG (SEQ ID NO:48), residues 1-20 of human MELK). The specific AMPKα1 antibody employed in Figures 27, 28B and 29 was raised against the peptide (CTSPPDSFLDDHHLTR (SEQ ID NO:49), residues 344-358 of rat AMPKα1) whilst the antibody recognising both AMPKα1 and AMPKα2 employed in Fig 28A, was raised against the peptide (CDPMKRATIKDIRE (SEQ ID NO:50) residues 252 to 264 or rat AMPKα1). The anti MARK2 and MARK3 peptide antibodies we raised were not specific as they immunoprecipitated other MARK isoforms as well. The anti MARK3 antibody from Upstate Biotech (anti c-TAK #05-680), raised against the human MARK3 protein was found to immunoprecipitate MARK2 as efficiently as MARK3 but did not immunoprecipitate MARK1 and MARK4 (data not shown). The Phosphospecific antibodies recognising AMPK phosphorylated on the T-loop were generated against as described previously

against the peptide (KFLRT(P)SCGSPNYA (SEQ ID NO:111) residues 168 to 180 of rat AMPKα1) (Sugden et al., 1999).The LKB1 antibody used for immunoblotting was raised in sheep against the mouse LKB1 (Sapkota et al., 2001) and that for immunoprecipitation was raised in sheep against the human LKB1 protein (Boudeau et al., 2003). Monoclonal antibody recognizing the HA epitope tag was from Roche, monoclonal antibodies recognising the GST and the FLAG epitopes were from Sigma. Anti-*myc* antibodies were prepared by ammonium sulphate precipitation of medium from Myc1-9E10 hybridoma cells grown in RPMI 1640 medium supplemented with 2 mM glutamine and 15% (v/v) foetal bovine serum. Secondary antibodies coupled to horseradish peroxidase used for immunoblotting were obtained from Pierce.

Please cancel the paragraph bridging pages 140 and 141, and, in its place, add the following new paragraph:

Cloning of AMPK-related kinases.

NUAK1. In order to obtain the coding region of human NUAK1 cDNA (NCBI Acc. NM_014840) a PCR reaction was carried out using a brain cDNA library (Clontech) as a template and the sense primer 5'- actgcagccctggagcccaggaagc-3' (SEQ ID NO:112) and the antisense primer 5'- ctagttgagc ttgctgcagatctccagcgc-3' (SEQ ID NO:113). The

resulting PCR product covered the coding region of NUAK1 from amino acid residues 31-661. The cDNA of the missing N-terminal 31 amino acids was incorporated into the 5' primer of another PCR reaction, then the HA tag was added by PCR using the sense primer 5'-actagtgccaccatgtaccc atacgatgtgccagattacgccgaaggggccgccgcgcctgtggcg ggg-3' (SEQ ID NO:114) and antisense primer 5-ctagttgagcttgctgcagatctccagcgc-3' (SEQ ID NO:115). The resulting PCR product was ligated into pCR2.1-TOPO vector (Invitrogen), and subcloned as a *Spe1-Not1* fragment into pEBG2T (Sanchez et al., 1994) and as an *EcoR1-EcoR1* insert into pGEX6P-1 (Amersham) expression vectors.

Please cancel the paragraph at page 141, lines 9-17, and, in its place, add the following new paragraph:

NUAK2. The coding region of human NUAK2 cDNA (NCBI Acc. NP_112214) with an N-terminal HA tag, was amplified by PCR from IMAGE consortium EST clone 6718982 (NCBI Acc. CA487493) with primers 5'-actagtgccaccatgtacccatacgatgtgccagattacgcc gagtcgctggttttcgcgcggcgctcc-3' (SEQ ID NO:116) and 5'- tcaggtgagctttgagcagaccctcagtgcctg-3' (SEQ ID NO:117). The resulting PCR product was ligated into pCR2.1-TOPO vector, sequenced, and subcloned as a *Spe1-Spe1* fragment into pEBG2T

and as an *EcoR1-EcoR1* insert into pGEX6P-1 expression vectors.

Please cancel the paragraph at page 141, lines 18-27, and, in its place, add the following new paragraph:

QIK. The coding region of human QIK cDNA (NCBI Acc. XM_041314) with an N-terminal HA tag was amplified from IMAGE consortium EST clone 5495545 (NCBI Acc. BM799630) using sense primers 5'-gcgtcgactacccatacgatgtgccagattacgccgtcatggcgg atggcccgag-3' (SEQ ID NO:118) or 5'-gcactagttacc catacgatgtgccagattacgccgtcatggcggatggcccgag-3' (SEQ ID NO:119) for subsequent cloning into vectors pGEX6P-3 and pEBG2T respectively, and antisense primer 5'-gagcggccgctaattcaccaggacatac ccgttgtg-3' (SEQ ID NO:120). Amplified PCR products were ligated into pCR2.1 TOPO vector, sequenced, and subcloned as a *Sal1-Not1* or *Spe1-Not1* insert into pGEX6P-3 or pEBG2T respectively.

Please cancel the paragraph at page 142, lines 1-7, and, in its place, add the following new paragraph:

SIK. The coding region of human SIK cDNA (NCBI Acc. NM_173354) with an N-terminal HA tag was amplified from IMAGE consortium EST clone

4831049 (NCBI Acc. NM_173354) using primers 5'-gcggatcctacccatacgatgtgccagattacgccgttatcatgtcgg agttcagcgcgg-3' (SEQ ID NO:121) and 5'-gagcggccg ctcactgcaccaggacaaacgtgcc-3' (SEQ ID NO:122). The PCR product was ligated into pCR2.1 TOPO vector, sequenced, and subcloned as a *BamH1-Not1* insert into pGEX6P-1 and pEBG2T.

Please cancel the paragraph at page 142, lines 8-14, and, in its place, add the following new paragraph:

MELK. The coding region of human MELK cDNA (NCBI Acc. NM_014791) with an N-terminal HA tag was amplified from IMAGE consortium EST clone 4547136 (NCBI Acc. BC014039) using primers 5'-gcggatcctacccatacgatgtgccagattacgccaaagattatgatg aacttctcaaatattatgaattacatg-3' (SEQ ID NO:123) and 5'- gtgcggccgcttataccttgcagctagataggatgt cttcc-3' (SEQ ID NO:124). The PCR product was ligated into pCR2.1 TOPO vector, sequenced, and subcloned as a *BamH1-Not1* insert into pGEX6P-1 and pEBG2T.

Please cancel the paragraph bridging pages 142 and 143,, and, in its place, add the following new paragraph:

BRSK1. Nucleotides 60-1010 of the coding region of human BRSK1 cDNA (NCBI Acc. NM_032430) were

amplified from IMAGE consortium clone 6154749 (NCBI Acc. BQ434571) using primers 1. 5'-ccacccc cacccaccccagcacgcccaatatgtgggcccctatcggctggagaag acgctgggcaaagg-3' (SEQ ID NO:125) and 2. 5'-cgatgcagcctctcgcggtccctgaagcagc-3' (SEQ ID NO:126), nucleotides 60-106 being added by primer 1. Nucleotides 980-2337 of BRSK1 were amplified from the same EST clone using primers 3. 5'-gctgcttcagggaccgcgagaggctgca tcg-3' (SEQ ID NO:127) and 4. 5'-tcagggcagaggggtcccgttggt ggcc-3' (SEQ ID NO:128). Each PCR product was ligated into pCR2.1 TOPO vector and sequenced. A single nucleotide difference compared with BRSK1 NM_032430 sequence that is present in the EST clone was 'corrected' by site-directed mutagenesis in the pCR2.1 TOPO clone containing the 5' half of BRSK1. The remaining 5' 59 nucleotides of BRSK1 and the HA tag were added to the 5' half of BRSK1 by PCR using sense primers 5. 5'- ggtgggggctctcccgcctaccacctcccccac ccccacccccacccacccccagcacgcccaatatg-3' (SEQ ID NO:129) and 6. 5'- ggatcctacccatacgatgtgccagatta cgcctcgtccggggccaaggagggaggtgggggctctcccgcctacc-3' (SEQ ID NO:130). The final PCR product was ligated into pCR2.1 TOPO vector and sequenced. Overlap PCR was then performed using the 5' half and 3' half of BRSK1 as templates and primer 7. 5'-gcggatcctacccatacgatgtgcc-3' (SEQ ID NO:131) and primer 4. and the PCR product ligated into pCR2.1 TOPO vector and sequenced. Full length

BRSK1 cDNA with N-terminal HA tag was then subcloned into pGEX6P-1 and pEBG2T as a *BamH1-BamH1* insert.

Please cancel the paragraph at page 143, lines 11-18, and, in its place, add the following new paragraph:

BRSK2. The coding region of HA-tagged human BRSK2 cDNA (NCBI Acc. AF533878) was amplified by PCR from IMAGE consortium EST clone 6144640 (NCBI Acc. BU193218) using primers 5'-ggatccgcca ccatgtacccatacgatgtgccagattacgccacatcgacggggaagg acggcggcgcg-3' (SEQ ID NO:132) and 5'-gcggccgctc agaggctactctcgtagctggtggccaccttctggcccttaagccca-3' (SEQ ID NO:133). The resulting PCR product was cloned into pCR2.1-TOPO vector, sequenced, and subcloned as a *BamH1-Not1* insert into pEBG2T and pGEX6P-1 vectors.

Please cancel the paragraph bridging pages 143, 144, and 145, and, in its place, add the following new paragraph:

QSK. Nucleotides 55-640 of the coding region of human QSK cDNA (sequence obtained from Sugen database www.kinase.com (Manning et al., 2002)) were amplified from IMAGE consortium EST clone 4396995 (NCBI Acc.BF983268) using primers 1. 5'-ggagccgggcccgcgggccgcctgctgcctccgcccgcgccggggtcc

ccagccgcccccgctgccgtgtcccctgcggccggccagccg-3' (SEQ ID NO:134) and 2. 5'-tgaagaggttactgaaaccaaa atctgctattttgatattc-3' (SEQ ID NO:135), nucleotides 55-110 being added by primer 1. The remaining 5' 54 nucleotides and HA tag were subsequently added by PCR using primers 3. 5'-gattacgccgcggcggcggcggcgagcggagctggcggggctgccggg gccgggactgggggagccgggcccgcgggccgcctgctg-3' (SEQ ID NO:136) and 4. 5'-gcggatcctacccatacgatgtgccag attacgccgcggcggcggcggcgagcgg-3 (SEQ ID NO:137). The final PCR product was ligated into pCR2.1 TOPO vector and sequenced to produce pCR2.1 QSK clone 1. Nucleotides 610-865 of QSK were amplified from IMAGE consortium EST clone 6247938 (NCBI Acc. BQ685213) using primers 5. 5'-atagcagattttggtttcagtaacctcttcactcctgggcagctg ctgaagacctggtgtggcagccctccctatgctgcacctgaactc-3' (SEQ ID NO:138) and 6. 5'- ctgtggacataaaaaatggga tgcggaactttcc-3' (SEQ ID NO:139), nucleotides 610-674 being added by primer 5. The PCR product was ligated into pCR2.1 TOPO vector and sequenced. A single nucleotide deletion at QSK open reading frame position 696, which is present in the EST clone, was corrected by site-directed mutagenesis to produce pCR2.1 QSK clone 2. QSK PCR products from pCR2.1 QSK clones 1 and 2 were input into an overlap PCR using primers 4. and 6. This product (QSK nucleotides 4-865 with N-terminal HA tag) was ligated into pCR2.1 TOPO vector and sequenced to produce

pCR2.1 QSK clone 3. QSK nucleotides 832-4110 were amplified from IMAGE consortium EST clone 360441 (NCBI Acc. AA015726) using primers 7. 5'-ggaaagttccgcatcccatttttatgtccacag-3' (SEQ ID NO:140) and 8. 5'-gagcggccgcttacacgcctgcct gctcca tgc-3' (SEQ ID NO:141). The PCR product was ligated into pCR2.1 TOPO vector and sequenced to produce pCR2.1 QSK clone 4. QSK PCR products from pCR2.1 clones 3 and 4 were input into an overlap PCR using primers 4. and 8. to generate full length QSK cDNA with N-terminal HA tag. The PCR product was ligated into pCR2.1 TOPO vector and sequenced to produce pCR2.1 QSK clone 5. Full length QSK with N-terminal HA tag was subcloned from pCR2.1 QSK clone 5 as a *BamH1-BamH1* insert into pGEX6P-1 and pEBG2T vectors. In order to generate the T-loop mutations in QSK, mutagenesis was performed on pCR2.1 QSK clone 3, followed by overlap PCR to generate the full length mutant forms of QSK. The overlap PCR product was ligated into pCR2.1 TOPO, sequenced, and subcloned into pGEX6P-1 and pEBG2T as a *BamH1-BamH1* insert.

Please cancel the paragraph at page 145, lines 7-21, and, in its place, add the following new paragraph:

MARK1. Nucleotides 4-1078 of the coding region of human MARK1 cDNA (NCBI Acc. NM_018650) with

an N-terminal HA tag were amplified from a human brain cDNA library using primers 5'- gcgaattctac ccatacgatgtgccagattacgcctcggcccggacgccattgc-3' (SEQ ID NO:142) and 5'-catcatacttctgatttattaaggc atcatttatttc-3' (SEQ ID NO:143). Nucleotides 1042-2388 of MARK1 were amplified from IMAGE consortium EST clone 48109 (NCBI Acc. H11850) using primers 5'- gaaataaatgatgccttaataaatcagaag tatgatg-3' (SEQ ID NO:144) and 5'-gagtcgacttacag cttaagctcatttgctatttttgatgc-3' (SEQ ID NO:145). Amplified PCR products were input into an overlap PCR to generate the full length HA-tagged MARK1 cDNA. The PCR product was ligated into pCR2.1 TOPO vector, sequenced, and subcloned as an *EcoR1-Sal1* insert into pGEX6P-1. Full length HA-tagged MARK1 was amplified from the pCR2. 1 TOPO MARK1 clone for subsequent cloning into pEBG2T as a *Kpn1-Not1* insert using primers 5'-gcggtacctacccatacgatgtgccagattacgcctc ggcccggacgccattgc-3' (SEQ ID NO:146) and 5'-gagcggccgcttacagcttaagctcatttgctatttttgatgc-3' (SEQ ID NO:147).

Please cancel the paragraph bridging pages 143, 144, and 145, and, in its place, add the following new paragraph:

MARK2. The coding region of human MARK2 cDNA (NCBI Acc. NM_004954) was amplified from IMAGE consortium EST clone 4591688 (NCBI Acc.

BG419875) using sense primers 5'-gcgtcgactacccat acgatgtgccagattacgccattcggggccgcaactcagcc-3' (SEQ ID NO:148) or 5'-gcactagttacccatacgatgtgcca gattacgccattcggggccgcaactcagcc-3' (SEQ ID NO:149) for subsequent cloning into vectors pGEX6P-3 and pEBG2T respectively, and antisense primer 5'-gagcggccgcttaaagcttcagctcgttggctattt tgg-3' (SEQ ID NO:150). Amplified PCR products were ligated into pCR2.1 TOPO vector, sequenced, and subcloned as a *Sal1-Not1* or *Spe1-Not1* insert into pGEX6P-3 or pEBG2T vectors respectively.

Please cancel the paragraph at page 145, lines 5-11, and, in its place, add the following new paragraph:

MARK3. The coding region of human MARK3 cDNA (NCBI Acc. NM_002376) with an N-terminal HA tag was amplified from IMAGE EST 5104460 (NCBI Acc. BI223323) using primers 5'- gcggccgcagccaccatgta cccatacgatgtgccagattacgcctccactaggaccccattgccaac ggtga-3' (SEQ ID NO:151) and 5'-gcggccgcttacagct ttagctcattggcaattttggaagc-3' (SEQ ID NO:152). The resulting PCR product was cloned into pCR2.1-TOPO vector, sequenced, and subcloned as a *Not1-Not1* insert into pEBG2T and pGEX6P-2 vectors.

Please cancel the paragraph at page 145, lines 12-25, and, in its place, add the following new paragraph:

MARK4. In order to obtain the full length coding region of human MARK4 cDNA (NCBI Acc.AK075272) two EST clones were used as templates for PCR reactions. The first 228 amino acids with an N-terminal HA tag was amplified from IMAGE consortium EST clone 6301902 (NCBI Acc.BQ709130) using primers 1. 5'- agatctgccaccatgtacccatacgat gtgccagattacgcctcttcgcggacggtgctggccccggg-3' (SEQ ID NO:153) and 2. 5'- tgccctgaaacagctccgggg cggc-3' (SEQ ID NO:154). The remaining coding region was amplified from IMAGE consortium EST clone 5503281 (NCBI Acc. BM467107) with primers 3. 5'-gggatcgaagctggacacgttctgc-3' (SEQ ID NO:155) and 4. 5'- gcggccgctcacactccaggggaatcgg agcagccgggg-3 (SEQ ID NO:156). The resulting PCR products were used as templates in an overlap PCR reaction with primers 1. and 4. The PCR product was ligated into pCR2.1-TOPO, sequenced, then subcloned further as a *Bgl2-Not1* insert into the *BamH1-Not1* site of pEBG2T and pGEX6P-1 vectors.

Please cancel the paragraph bridging pages 149 and 150, and, in its place, add the following new paragraph:

Measurement of activation of AMPK-related kinase.s AMPKα1 catalytic subunit and AMPK-related kinases was measured following their phosphorylation with LKB1 as follows. 1-2 μg of AMPKα1 catalytic domain or AMPK-related kinase, were incubated with or without 0.1-1 μg of wild type of the indicated LKB1 complex in Buffer A containing 5 mM MgAcetate and 0.1 mM ATP, in a final volume of 20 μl. After incubation at 30°C for the times indicated in the figure legend, AMPKα1 catalytic domain or AMPK-related kinase activities were determined by adding 30 μl of 5 mM magnesium acetate, 0.1 mM [γ-^{32}P]-ATP (300 cpm/pmol) and 200 μM *AMARA* peptide (AMARAASAAALARRR (SEQ ID NO:157) (Dale et al., 1995)) as substrate . After incubation for 5-20 min at 30°C, incorporation of ^{32}P-phosphate into the peptide substrate was determined by applying the reaction mixture onto P81 phosphocellulose paper and scintillation counting after washing the papers in phosphoric acid as described previously (Alessi et al., 1995). One Unit (U) of activity was defined as that which catalysed the incorporation of 1 nmol of ^{32}P into the substrate. Time course reactions for the second stage of the assay were performed in order to ensure that the rate of phosphorylation was occurring linearly with time. Kinetic data was analysed according to the Michaelis-Menten relationship by non-linear regression using the

computer program GraphPad Prism (GraphPad Software Inc, San Diego, USA).

Please cancel the paragraph bridging pages 152 and 153, and, in its place, add the following new paragraph:

Immunoprecipitation and assay of endogenous LKB1 employing LKBtide substrate.
0.1-1 mg Hela or MEF cell lysate protein was incubated at 4°C for 1h on a shaking platform with 5 μl of protein G-Sepharose conjugated to 5 μg of human LKB1 antibody. The immunoprecipitates were washed twice with 1 ml of Lysis Buffer containing 0.5 M NaCl, and twice with 1 ml of Buffer A. Phosphotransferase activity towards the LKBtide peptide (LSNLYHQGKFLQTFCGSPLYRRR (SEQ ID NO:158) residues 241-260 of human NUAK2 with 3 additional Arg residues added to the C-terminal to enable binding to P81 paper), was then measured in a total assay volume of 50 μl consisting of 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 0.1% (by vol) 2-mercaptoethanol, 10 mM magnesium acetate, 0.1 mM [γ^{32}P]ATP (~200 cpm/pmol) and 200 μM LKB1tide peptide. The assays were carried out at 30°C with continuous shaking, to keep the immunoprecipitates in suspension, and were terminated after 10 min by applying 40 μl of the reaction mixture onto p81

membranes. The p81 membranes were washed in phosphoric acid, and the incorporated radioactivity was measured by scintillation counting as described previously for MAP kinase (Alessi et al., 1995).

Please cancel the paragraph on page 163, line 1 with the following new paragraph:

WE CLAIM: